Together, Taking on Cancer’s Toughest Challenges

UTILIZING GENETIC MODELS FOR CARDIO-ONCOLOGY: GETTING TO THE HEART OF THE MATTER

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Medical College of Wisconsin
Radiation-Induced Cardiotoxicity

- Radiation therapy received by >50% of all cancer patients
  - Includes breast cancer patients; >3.5 million U.S. breast cancer survivors in U.S.

- Radiation improves cancer-specific survival, but heart radiation can cause long-term toxicity

- Relative risk of cardiovascular events in early left-sided breast cancer can be 1.3 or greater
  - Higher risk with other cardiac risk factors, with cardiotoxicity systemic therapy, and higher doses due to involved lymph nodes
  - Linear relationship between mean heart dose and major cardiovascular events
RADIATION-INDUCED CARDIOTOXICITY

- Relative risk of fatal cardiovascular events is 2-7 in Hodgkin’s Lymphoma patients who receive radiation.
- Relative risk of fatal cardiovascular events is 2-6+ in childhood cancer survivors receiving higher cardiac radiation doses.

mcw.edu/departments/cancer-center @MCWCancerCenter
Radiation-induced cardiotoxicity

• Radiation also plays an integral role in treating advanced lung cancer

• Recent studies show that radiation-induced cardiotoxicity could manifest within 2 years of radiation for lung cancer

• In lung cancer patients, mortality correlates with mean heart dose, or with the percent of the heart receiving 5 Gy, 30 Gy, or 50 Gy
GOAL: IMPROVE THERAPEUTIC RATIO

Mcw.edu/departments/cancer-center  @MCWcancerCenter  Adapted from https://www.healthxchange.sg/
STRATEGIES TO MINIMIZE RADIATION-INDUCED CARDIOTOXICITY

- Mounting evidence suggests that complex genetic modifiers contribute to the risk of radiation-induced toxicity in cancer patients
  - These genetic modifiers remain largely unknown and poorly understood

- We have developed the first genetic model to identify heritable modifiers of radiation-induced cardiotoxicity
Aim to identify factors that modify normal tissue side effects
RESEARCH PLAN

Cardiac Irradiation in Genetic Animal Models

Aim to identify factors that modify normal tissue side effects
Data suggested differences in the Sprague Dawley (SD) and Brown Norway (BN) rat strain responses to heart radiation.

The PhysGen Database from MCW (pga.mcw.edu) gives physiologic and pathologic parameters for SS (derived from SD) and BN inbred strains.

Cardiac phenotypes differed between SS and BN rat strains.
**SS AND BN RAT CARDIAC PHENOTYPES**

- Differences in SS and BN rats for post-ischemic infarct size

![Post-Ischemic Infarct Size](chart.png)

- Start by testing SS vs. BN radiation responses
- If differences in cardiac radiation sensitivity, consomic rat testing (chromosome substitution) may be used to identify genetic variants

*From pga.mcw.edu*
RAT MODEL TO TEST CARDIAC RADIATION SENSITIVITY

• Treated to 24 Gy with 3 equal fields, AP and 2 laterals, with 1.5 cm circular field

• Echocardiograms with strain analysis performed to assess cardiac function
  • Baseline
  • 3 months
  • 5 months
RAT MODEL TO TEST CARDIAC RADIATION SENSITIVITY
• Adult female rats given 24 Gy localized cardiac radiation

• Hearts harvested after 5 months

• SS rats had significant cardiac hypertrophy (heart enlargement), while BN rats did not
  • Hypertrophy occurs to compensate for poorer function
  • Consistent with hypothesis
  • Consomic studies pursued

Reviewed consomic tumor studies and PGA to guide which consomic rats to initially test
CONSOMIC RAT MODEL: SS.BN3

- Consomic rats
  - Rats with same genetic makeup except one chromosome substituted from another strain
SS/BN/SS.BN3 RAT CARDIAC PHENOTYPES

- Differences in SS and BN post-ischemic infarct size

Start by testing SS vs. SS.BN3 consomic cardiac radiation responses

**Hypothesis:** SS and SS.BN3 rats will respond differently to heart radiation

*From pga.mcw.edu*
CONSOMIC RAT MODEL TO TEST CARDIAC RADIATION SENSITIVITY

- Assess SS.BN3 versus SS normal heart tissue radiation sensitivity
- Start with 24 Gy x 1
- Obtain echocardiograms at 0, 3, and 5 months
SS RAT HEARTS MORE SENSITIVE TO RADIATION

• Amount of fluid in the lungs (pleural effusions) increased in the SS rats after radiation
  • Fluid can be present due to inflammation or heart failure

![Graph showing pleural fluid levels in SS and SS.BN3 rats over 2.5 and 5 months.](image)

*P < 0.05
SS RATS EXPERIENCE MORTALITY AFTER LOCALIZED CARDIAC RADIATION

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**Graph:**

- **Y-axis:** Percent survival
- **X-axis:** Time

**Legend:**
- SS (n=11)
- SS.BN3 (n=7)

- 0/7
- 5/11

*P < 0.05
NO SIGNIFICANT DIFFERENCES IN ECHOCARDIOGRAMS FROM UNTREATED RATS
SS RA T HEARTS EXHIBIT SYSTOLIC DYSFUNCTION VIA ECHOCARDIOGRAM

• The SS heart exhibits decreased contractility after radiation
SS RA T HEA RTS EXHIBIT SYSTOLIC DYSFUNCTION VIA ECHOCARDIOGRAM

- Echocardiogram data

![Graph showing End Systolic Volume over time after radiation]

- Time After Radiation
- 0 Mo, 3 Mo, 5 Mo
- End Systolic Volume (ESV ml/kg)
- SS and SS.BN3 groups
- †P < 0.01
SS RATS’ HEARTS EXHIBIT SYSTOLIC DYSFUNCTION VIA ECHOCARDIOGRAM

- Echocardiogram data

**End Systolic Volume**

- **SS**
- **SS.BN3**

**Time After Radiation**

- 0 Mo
- 3 Mo
- 5 Mo

**Ejection Fraction**

- **SS**
- **SS.BN3**

**Months Post-RT**

- 0
- 1
- 3
- 5

† *P < 0.01*
SS Rats Have Lower Strain on echocardiogram.

Left Ventricular Radial Strain

SS.Rats have lower strain on echocardiogram.

<table>
<thead>
<tr>
<th>Time After Radiation</th>
<th>Thickening of Myocardium (%)</th>
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</thead>
<tbody>
<tr>
<td>0 Mo</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>3 Mo</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>5 Mo</td>
<td>60 ± 10</td>
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</tbody>
</table>

†P < 0.01
#P < 0.001
INCREASED MYOCARDIAL NECROSIS IN SS RATS 5 MONTHS AFTER RADIATION
INCREASED FIBROSIS IN SS RATS 5 MONTHS AFTER RADIATION

TRICHOROME STAINING

SS

SS.BN3
SS RATS HEARTS MORE SENSITIVE TO 5X9 GY FRACTIONATED RADIATION

Male rats also exhibit similar patterns, with SS more sensitive than SS.BN3
HEART GENE EXPRESSION CHANGES AFTER RADIATION

- RNA-seq gene expression data
  - From left ventricle at 1 week after radiation
CARDIOTOXICITY FROM A VARIETY OF CANCER THERAPIES

• Chemotherapeutic and targeted therapies can cause cardiac dysfunction
  • Doxorubicin and other anthracyclines have dose-dependent cardiac risk

• Many breast cancer and lymphoma patients receive radiation heart exposure after receiving doxorubicin
  • Doxorubicin and radiation cause higher risk of cardiac dysfunction than single modality treatment

• Are the SS rats more sensitive to doxorubicin-induced cardiotoxicity?
SS RATS MORE SENSITIVE TO DOXORUBICIN THAN SS.BN3 RATS

• Adult female rats treated with doxorubicin
  • 2 mg/kg weekly I.P. for 8 weeks; causes heart changes similar to clinical changes

• Echocardiograms revealed worse cardiac function in the SS rats, similar to cardiac radiation
  • Increased end diastolic volume (EDV) and end systolic volume (ESV) in SS rats after doxorubicin
SUMMARY AND NEXT STEPS

• Many distinct measures of cardiac damage are worse in SS vs. SS.BN3 rats
  • Male and female rats
  • Both large single fraction and fractionated (9 Gy x 5)

• Utilize genetic mapping to identify variants in chromosome 3 enhancing radiosensitivity
CONGENIC MAPPING

- Narrow down region of interest on rat chromosome 3
CONGENIC RAT PANEL

- Adult female rats given 24 Gy localized cardiac radiation
- Hearts harvested after 5 months
- Echocardiograms with strain analysis performed at 0, 3, and 5 months
QUESTION:
Which of the SS.BN3 congenic rats will respond similarly to SS with respect to cardiac irradiation?
SS AND SS.BN3 + CONGENIC RATS

SS heart

 Radiation

 Radiation

 Radiation

 Radiation

 SS.BN3 heart

 CG.1 heart

 Radiation

 CG.2 heart

 Radiation

 CG.3 heart

 Radiation

 CG.4 heart

 Radiation

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 ?
CG.4 RATS HAVE SIMILAR NORMALIZED HEART WEIGHT TO SS RATS POST-RT

<table>
<thead>
<tr>
<th># Rats treated per group (RT)</th>
<th>SS</th>
<th>SS.BN3</th>
<th>CG.1</th>
<th>CG.2</th>
<th>CG.3</th>
<th>CG.4</th>
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<tr>
<td></td>
<td>9</td>
<td>10</td>
<td>14</td>
<td>14</td>
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</tbody>
</table>

*P<0.01

**P<0.001
CG.4 have increased pleural effusions similar to SS rats post-radiation.
Left Ventricular Radial Strain

Sign. Diff @ 0 mo?
- SS.BN3 to SS: No
- CG.1 to SS: No
- CG.2 to SS: No
- CG.3 to SS: No
- CG.4 to SS: No

Sign. Diff @ 3 mo?
- SS.BN3 to SS: Yes
- CG.1 to SS: Yes
- CG.2 to SS: Yes
- CG.3 to SS: Yes
- CG.4 to SS: No

Sign. Diff @ 5 mo?
- SS.BN3 to SS: Yes
- CG.1 to SS: No
- CG.2 to SS: Yes
- CG.3 to SS: Yes
- CG.4 to SS: No

CG.4 RATS HAVE DECREASED RADIAL STRAIN SIMILAR TO SS RATS POST-RADIATION
CONGENIC MAPPING CONCLUSIONS THUS FAR

- 25 MB region contains 331 genes, 131 expressed genes, and 93 genes differentially expressed after RT
Differentially Expressed Genes in the 25 Mb Region

<table>
<thead>
<tr>
<th>Heart</th>
<th>Mitochondria</th>
<th>Notch</th>
<th>Other</th>
<th>Apoptosis/Autophagy</th>
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<tbody>
<tr>
<td>Pygb</td>
<td>Jph2</td>
<td>Slc35c2</td>
<td>Epb41l1</td>
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<td>Csnk2a1</td>
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<td>Uqcc1</td>
<td>Pofut1</td>
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<td>Ndufaf5</td>
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<td>Acss2</td>
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2019 Scientific Retreat – Together, Taking on Cancer’s Toughest Challenges
TESTING CANDIDATE PATHWAYS

- The Sirt3 and mitochondrial function pathways were top differentially expressed pathways in the SS vs. SS.BN3 hearts after radiation
  - We are examining enhancing these pathways to protect against radiation
- Honokiol
  - Used in Chinese medicine > 1,000 years
  - Active extract from magnolia bark
  - Activator of Sirt3
  - Preserves mitochondria and protects doxorubicin-induced cardiomyopathy in mice

C Koentges et al., 2016 Front Cardiovasc Med

TESTING WHETHER HONOKIOL PROTECTS AGAINST RADIATION

• Mito-honokiol targets honokiol to the mitochondria
  • Mito-honokiol shows enhanced activity to inhibit cancer cell growth (Ming You, MD, PhD & Balaraman Kalyanaraman, PhD)

Hypothesis: Honokiol protects rats against radiation-induced cardiotoxicity, and mito-honokiol will protect to a greater extent

This will be tested later this year

Pan et al., iScience 2018
SUMMARY AND CONCLUSIONS

• Genetic variants on rat chromosome 3 are important for cardiac radiation injury
  • Also true for doxorubicin injury
  • Mitochondrial- and Sirtuin-related pathways may be important

• First genetic model of radiation-induced cardiotoxicity

• Consomic panels used to identify genetic variants influencing radiation and other treatment toxicities
  • Also identifies critical downstream pathways
WHY I AM A CANCER RESEARCHER AND PHYSICIAN

• My uncle (died age 41, colon cancer)
• My aunt (breast cancer, age 44)
• My grandmother (breast cancer, age 80)
• My uncle (prostate cancer, age 57)
• My great aunt (breast cancer, age 74)
• My cousin (breast cancer, age 46)
• My cousin (prostate cancer, age 50)
• My close friend (multiple myeloma, age 63)
• Another friend (died age 40, breast cancer)
• My patients (too many to count)
• Future family members and friends affected by cancer
BERGOM LAB

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If you want to go fast, go alone.

If you want to go far, go together.

– African Proverb

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